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## Specific immunization using keyhole limpet hemocyanin-ganglioside conjugates.

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In a search for adjuvants of non-bacterial origin for immunization with ganglioside, we investigated whether chemical coupling to immune stimulatory protein could increase the immunogenicity of sialoglycosphingolipid. A novel method for the linkage of glycosphingolipids, including gangliosides, to protein was established. The procedure includes lysis of the sphingoid double bond by ozone, reduction of the ozonolysis product to the aldehyde, and coupling to amino groups, either directly by reductamination, or by conjugation via a long aliphatic chain dicarboxylic acid linker. Using this method, gangliosides Gfpt1 (IV2-Fuc-, II3NeuAc-Gg4Cer), Glac2 [II3(NeuAc)2-LacCer], and Gtet1 (II3NeuAc-Gg4Cer) were coupled to keyhole limpet hemocyanin (KLH), and the immunogenicity of the conjugates was tested. Immunization of mice with the KLH-ganglioside conjugates led in each case to the formation of IgG- and IgM antibodies that recognized the underivatized gangliosides, respectively. In contrast to this, mixtures of KLH and ganglioside proved ineffective for immunization. KLH-tumor-associated ganglioside conjugates may, therefore, be considered as possible vaccines in immune therapy of cancer.

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